

## CLAIMS

1. Method to detect and phenotype target cells, such as animal and human cells, in cell suspensions by using particles coated with antibodies/ligands directed against antigenic determinants/receptors expressed on the target cells, except when the target cells are malignant and normal haematopoietic and lymphatic cells, characterized in that 2 - 6 antibodies or ligands, each antibody or ligand conjugated to each of several types of particles instrumentally or visually separable by fluorescence, color and size, with sizes ranging from 0.01  $\mu\text{m}$  - 6  $\mu\text{m}$ , wherein the ratio between the number of particles and the number of cells ranges from 20 : 1 to 0.5 : 1, are incubated under gentle rotation for 5-10 minutes to 2 hours with cell suspensions containing the target cells at 0°C to 25°C, optionally followed by a per se known enrichment procedure, and evaluation of the target cell rosettes microscopically and/or by suitable visualizing or imaging devices.

2. Method according to claim 1, characterized in that the said size of the particles ranges from 0.5  $\mu\text{m}$  - 4.5  $\mu\text{m}$ , the said ratio is 5 : 1 (number of particles/number of cells), the said incubation time is 30 minutes and the said incubation temperature is 4 ° C

3. Method according to claim 1 and 2, characterized in that the particles used in the method are separable by a combination of fluorescence and/or size or a combination of fluorescent emission spectra, different colors or different sizes.

4. Method according to claim 3, characterized in that the particles used are separable by a combination of fluorescent emission spectra and/or size.

5. Method according to claim 1-4, characterized in that the particles used in the method are coated with ligands/antibodies directed against adhesion molecules, carbohydrate antigens, glycolipids, growth factor receptors, melanoma antigens, sarcoma antigens, carcinoma markers, neuroblastoma antigens, glioma antigens, head and neck cancer antigens, apoptosis-associated molecules, motility-related antigens, proliferation-associated antigens, differentiation-associated

Sub  
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MC2

a

a

antigens, drug resistance-related antigens, angiogenesis-associated antigens, chemokine receptors, invasion-related antigens, cathepsin D, neuroglandular antigen and pan-human antigen.

5 6. Method according to claim 5, characterized in that the particles used in the method are coated with ligands/antibodies directed against the receptors/antigens listed in Table 1.

7. Method according to <sup>claim 5</sup> ~~claims 5-6~~, characterized in that the particles used in the method are coated with antibodies directed to tumor associated antigens.

10 8. Method according to claim 7, characterized in that the tumor associated antigens are MOC31 anti EGP2 (anti-epithelial cell marker) antibody, anti-breast mucin (MUC1) antibody (BM7), 595, anti-EGF receptor (425.3), anti-erbB2 and anti-HMW melanoma antigen (9.2.27).

15 9. Use of the method according to claim 1-8, wherein it is performed phenotyping of the target cells comprising profiling the antigenic determinants or receptors expressed on the cell membrane of the target cells.

20 10. Use according to claim 9, wherein the target cell characteristics of biologically informative markers of diagnostic, prognostic and therapeutic value are registered.

11. Use according to claim 10, wherein the target cells are malignant cells.

25 12. Use according to claim 10, wherein the biologically informative markers are adhesion molecules, growth factor receptors, carcinoma markers, carbohydrate antigens, melanoma antigens, sarcoma antigens, glioma antigens, apoptosis associated markers, motility related markers, proliferation associated antigens, differentiation associated markers, drug resistance markers, angiogenesis associated markers chemokine receptors, invasion-related markers and other antigens.

30 13. Use according to claim 12, wherein the adhesion molecules are E-cadherin, the growth factor receptors are EGFr, c-erbB2, IL-2 receptor, TNF receptor, the carcinoma markers are EGP2, MUC1, MUC2 & 3, PSA, PSM, GA733.2, TAG72, 15-3 epitope, ovarian carcinoma CA- 125 epitope, the

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Ant  
B2a

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carbohydrate antigens are LeY, CEA, 15-3 epitope, the melanoma antigens are HMW 250000, gp 75/TRP-1, p95, MAG 1, 2, 3, the sarcoma antigens are TP 1 and TP 3 eptiopes, the glioma antigens such as Mel-14 epitope, apoptosis associated markers are Fas, FasL, p75, the motility related markers are KAT-1, AMF, the proliferation associated antigens are gp120, the differentiation associated markers are MUC 18, TA99, the drug resistance markers are MDR, MRP, the angiogenesis associated antigens are VEGFr, bFGF, the chemokine receptors are CCR, CXCR, the invasion-related markers are uPAR, uPA, PAI-1, TIMP1 & 2, MMP9, stromelysins, and the other antigens are cathepsin D and par-human epitope.

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14. Kit to perform the method according to ~~claim 1-8~~, characterized in in that it comprises particles conjugated to antibodies/ligands according to ~~claims 5-7~~.

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A C6md  
C7

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